Gonadal Function in Adult Male Patients with Congenital Adrenal Hyperplasia

Engels, Manon; Gehrmann, Katharina; Falhammar, Henrik; Webb, Emma A; Nordenstrom, Anna; Sweep, Fred; Span, Paul N; van Herwaarden, Antonius Eduard; Rohayem, Julia; Richter-Unruh, Annette; Bouvattier, Claire; Koehler, Birgit; Kortmann, Barbara B; Arlt, Wiebke; Roeleveld, Nel; Reisch, Nicole; Stikkelbroeck, Nike; Claahsen-van der Grinten, Hedi L

DOI:
10.1530/EJE-17-0862

Document Version
Peer reviewed version

Gonadal Function in Adult Male Patients with Congenital Adrenal Hyperplasia

Authors*:* M. Engels[1,2]; K. Gehrmann[3]; H. Falhammar[4,5]; E.A. Webb[6,7]; A. Nordenström[8]; F.C. Sweep[2]; P.N. Span[9]; A.E. van Herwaarden[2]; J. Rohayem[10]; A. Richter-Unruh[10]; C. Bouvattier[11]; B. Köhler[3]; B.B. Kortmann[12]; W. Arlt[6,7]; N. Roeleveld[13]; N. Reisch[14]; N.M.M.L. Stikkelbroeck[15]; H.L. Claahsen - van der Grinten[1] on behalf of the dsd-LIFE group[#].

Affiliations*:*

[1] Department of Pediatrics, Amalia Children's Hospital, Radboud university medical center, P.O. Box 9101, 6500HB Nijmegen, the Netherlands

[2] Department of Laboratory Medicine, Radboud Institute for Molecular Life Sciences (RIMLS), Radboud university medical center, P.O. Box 9101, 6500HB Nijmegen, the Netherlands

[3] Klinik für Pädiatrie m.S. Endokrinologie und Diabetologie, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Augustenburger Platz 1, 13353 Berlin, Germany

[4] Department of Endocrinology, Metabolism and Diabetes, Karolinska University Hospital, Stockholm, Sweden

[5] Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden

[6] Institute of Metabolism and Systems Research (IMSR), University of Birmingham, Birmingham, United Kingdom

[7]Centre for Endocrinology, Diabetes and Metabolism, Birmingham Health Partners, Birmingham, United Kingdom

[8] Department of women's and children's health, Division of Pediatric Endocrinology, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

[9] Department of Radiation Oncology, Radiotherapy & OncoImmunology laboratory, RIMLS, Radboud university medical center, Nijmegen, the Netherlands

[10] Centre of Reproductive Medicine and Andrology, Clinical Andrology, University Hospital Münster, Albert Schweitzer Campus 1; Building D 11, 48129 Münster, Germany

[11] Endocrinologie pédiatrique, Centre de référence des maladies rares du développement sexuel , Hôpital Bicêtre, , Université Paris-Sud, 78 rue du General Leclerc, 94270 Le Kremlin-Bicêtre, France

[12] Department of Pediatric Urology, Radboud university medical center, P.O. Box 9101, 6500HB Nijmegen, the Netherlands

[13] Department for Health Evidence, Radboud university medical center, P.O. Box 9101, 6500HB Nijmegen, the Netherlands

[14] *Medizinische Klinik IV,* Klinikum der Universität München, München, Germany

[15] Department of Internal Medicine, Radboud university medical center, P.O. Box 9101, 6500HB Nijmegen, the Netherlands

[#] Members of dsd-LIFE group are: Birgit Kohler, Berlin; Peggy Cohen-Kettenis and Annelou de Vries, Amsterdam; Wiebke Arlt, Birmingham; Claudia Wiesemann, Gottingen; Jolanta Slowikowska-Hilczer, Lodz; Aude Brac de la Perriere, Lyon; Charles Sultan and Francoise Paris, Montpellier; Claire Bouvattier, Paris; Ute Thyen, Lubeck; Nicole Reisch, Munich; Annette Richter-Unruh, Munster; Hedi Claahsen-van der Grinten, Nijmegen; Anna Nordenstrom, Stockholm; Catherine Pienkowski, Toulouse; and Maria Szarras-Czapnik, Warsaw

Corresponding author and person to whom reprint requests should be addressed:

Hedi Claahsen-van der Grinten, MD, PhD

Department of Pediatrics

Radboud university medical center (804)

P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

Phone: 0031-24-3614430

Fax: 0031-24-3616428

E-mail: Hedi.Claahsen@radboudumc.nl

Short title: Gonadal function in adult men with CAH

Keywords: congenital adrenal hyperplasia; gonadal function; fertility; testicular adrenal rest tumor; hypogonadism

Word count: ~~3593~~ 3779

Abstract

Context: Current knowledge on gonadal function in Congenital Adrenal Hyperplasia (CAH) is mostly limited to single center/country studies enrolling small patient numbers. Overall data indicate that gonadal function can be compromised in men with CAH. ~~Gonadal function can be compromised in male patients with congenital adrenal hyperplasia (CAH), however previous studies have been limited to reports from a single center/country or small patient numbers.~~

Objective: To determine gonadal function in men with CAH within the European "dsd-LIFE" cohort.

Design: Cross-sectional clinical outcome study, including retrospective data from medical records.

Methods: Fourteen academic hospitals included 121 men with CAH aged 16-68 years. Main outcome measures were serum hormone concentrations, semen parameters, and imaging data of the testes.

Results: At the time of assessment, ~~19/83~~ 14/69 patients had a serum testosterone concentration ~~level~~ below the reference range; ~~8~~ 7 of those were hypogonadotropic, ~~10~~ 6 normogonadotropic, and 1 hypergonadotropic. In contrast, ~~in the presence of~~ among the patients with normal serum testosterone (~~64/83~~ 55/69), ~~5~~ 4 ~~patients~~ were hypogonadotropic, ~~50~~ 44 normogonadotropic, and ~~9~~ 7 hypergonadotropic. The association of decreased testosterone with reduced gonadotropin concentrations (Odds Ratio (OR)=~~8.0 [2.2-29.6]~~ 12.8 [2.9-57.3]) was weaker than the association between serum androstenedione/testosterone ratio ≥ 1 and reduced gonadotropin concentrations (OR=~~16.8 [2.0-142.5]~~ 39.3 [2.1-732.4]). Evaluation of sperm quality revealed decreased ~~Decreased~~ sperm concentration~~s~~ (15/39), ~~decreased~~ motility (13/37), and abnormal morphology (4/28) ~~were also observed~~. Testicular adrenal rest tumor (TART)s were present in 39/80 patients, with a higher prevalence in patients with the most severe genotype (14/18), and in patients with increased current 17-hydroxyprogesterone (~~12/18~~ 20/35) or androstenedione (~~16/26~~ 12/18) serum concentrations. Forty-three children were fathered by 26/113 patients.

Conclusions: Men with CAH have a high risk of developing hypothalamic-pituitary-gonadal disturbances and spermatogenic abnormalities. Regular assessment of endocrine gonadal function and ~~of~~ imaging for TART development ~~by imaging~~ are recommended, in addition to measures for fertility protection.

Introduction

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder resulting in impaired adrenocortical steroid synthesis by several enzyme deficiencies. The most common form (>95%) is 21-hydroxylase deficiency (21OHD) with an incidence of 1:15 000, leading to glucocorticoid and often also mineralocorticoid deficiency in combination with androgen excess ~~ies~~ [1, 2].

Reported fertility and fecundity in men with CAH on routine steroid replacement therapy range from normal to severely impaired. Fertility can be compromised due to primary (hypergonadotropic) hypogonadism or central (hypogonadotropic) hypogonadism [3-11]. In addition, reduced fertility and fecundity rates ~~problems~~ in CAH can be caused by psychosexual factors [4]. ~~One of the commonest complications in men with CAH is the presence of Testicular Adrenal Rest Tumor (TART)s, which can cause disturbances of gonadal function, including mechanical obstruction of the seminiferous tubules. The reported prevalence of TART ranges between 12.5% and 94% of the populations studied~~. Central or secondary hypogonadism is defined as decreased testosterone concentrations in combination with either low or low-normal LH or FSH concentrations. In ~~patients~~ men with CAH, secondary hypogonadism is most likely ~~to be~~ caused by the suppressive effect of elevated adrenal androgens (that are aromatized to estrogens) on the hypothalamic-pituitary-gonadal (HPG)-axis [6]. Differentiation between gonadal and adrenal testosterone is difficult, complicating the diagnosis of hypogonadism in ~~patients~~ men with CAH. One of the commonest complications in men with CAH is the presence of Testicular Adrenal Rest Tumor (TART)s, which can cause disturbances of gonadal function, including mechanical obstruction of the seminiferous tubules. The reported prevalence of TARTs ranges between 12.5% and 94% in the populations studied [4-10, 12-22].

Until now, the data on fertility outcome in men with CAH are scarce~~. Available data are~~ [3-11] and often derived from studies with patients from a single center or country. Our aim was to study gonadal function in a large European multi-center cohort of male patients with CAH by evaluating hormone concentrations, semen parameters, and TART frequency.

Subjects and Methods

Subjects

dsd-LIFE is a cross-sectional clinical outcome study of individuals with disorders/differences of sex development (DSD). Fourteen study centers in 6 European countries (France (n=4), Germany (n=4), United Kingdom (n=1), Poland (n=2), Sweden (n=1), and the Netherlands (n=2)) included former and current patients as participants from February 2014 - September 2015. In addition to DSD participants, 121 male participants with CAH (46XY karyotype) aged 16-68 years were recruited as they may face similar clinical challenges as DSD patients, including sex hormone imbalances and fertility problems, although male patients with CAH do not fit into the classification of DSD. Written informed consent was obtained from all participants and/or their parents, with assent of minors. Ethical approvals were obtained as appropriate for each country~~, e.g. Ethics Commission of the Charité Universitätsmedizin; reference number EA2/069/13. For.~~ The theoretical and methodological framework of the dsd-LIFE study have been published in detail elsewhere ~~see Röhle 2017 et al.~~ [23]. Patients were investigated in their local treatment center. Cross-sectional data were obtained for serum hormone concentrations, semen parameters and testicular imaging. The genotype of patients with 21OHD was classified into genotype groups null, A, B, and C [24]. ~~Patients were also classified into salt-wasting (SW), simple virilizing (SV) or non-classical (NC) based on their main symptoms and time of diagnosis~~ General patient characteristics and clinical parameters included: country of inclusion, age, age at diagnosis, CAH genotype and phenotype, socioeconomic status, and obesity, as well as height, weight, and BMI throughout the years (at diagnosis, 9 months old, 6 years old, Tanner stage 2, 16 years old, and current age). Patients' educational levels was established according to the EU classification. We combined the standardized ES-ISCED (international standard classification of education) scale to Low (ES-ISCED I = less than lower secondary and ES-ISCED II = lower secondary); medium (ES-ISCED IIIb = lower tier upper secondary; ES-ISCED IIIA = upper tier upper secondary; ES-ISCED IV = advanced vocational, sub-degree) and high (ES-ISCED V1 = lower tertiary education, BA

level; ES-ISCED V2 = higher tertiary education, >=MA level). Data was collected during medical examination at study inclusion (cross-sectional) and retrieved from medical records (retrospective data).

Hormonal analysis

Blood samples were taken during day time, but mostly in the morning, before intake of the glucocorticoid medication [23]. Total testosterone, SHBG, LH, FSH, inhibin B, AMH, androstenedione, 17-hydroxyprogesterone (17OHP) concentrations, and renin/plasma renin activity were measured in the local hospital laboratory and compared to local references. Values are reported in SI or international units and reported as “below reference range”, “within reference range”, “above reference range up to twice the upper limit", and "more than twice the upper limit of the reference range”. To increase the number of patients per category, we combined the latter 2 categories into the category "above reference range".

The serum androstenedione/testosterone ratio (AD/T) was calculated and divided into normal (<0.5; interpreted as testosterone mainly of testicular origin), ≥0.5 and <1 (significant fraction of testosterone is of adrenal origin), and ≥1 (testosterone mainly of adrenal origin) as suggested by others [25].

~~Three patients were excluded from part of the analyses as they received testosterone substitution, which directly affects testosterone and gonadotropin concentrations. Two of these patients had data on TART available; these are described in the results section, but were otherwise excluded from further analyses.~~

Semen analysis

Semen analysis was performed by the local hospital laboratory and interpreted in accordance with the 2010 World Health Organization criteria [26], including sperm concentration (lower reference limit

(LRL): 15x10^6/mL) , motility (LRL: 40%), morphology (LRL: 4%), vitality (LRL: 58%), and volume (LRL: 1.5 mL).

Imaging of testes

At the study visit, 68 patients (56.2%) underwent testicular ultrasound. The presence of TART at the age of 16 years was also reported retrospectively (in 30/68 patients with cross-sectional TART data). In addition, retrospective data were available for 12 participants based on ultrasound findings or MRI (n=11) and on histological findings (n=1).

Paternity

Data about paternity and relationships were collected from the dsd-LIFE questionnaires [23].

Medication and estimation of metabolic control in the past

Patients used different formulations of glucocorticoids, including hydrocortisone, prednisone, prednisolone, and dexamethasone. Furthermore, we converted all ~~All~~ glucocorticoid preparations ~~were converted~~ to hydrocortisone equivalents ~~for comparison~~, using the following factors for the glucocorticoid equivalent dose: 1 (hydrocortisone), 4 (prednisone or prednisolone), 30 (dexamethasone), and 15 (fludrocortisone)[27]. We also calculated mineralocorticoid equivalent dose using the following factors: 1 (hydrocortisone), 0.8 (prednisone or prednisolone), 0 (dexamethasone), and 200 (fludrocortisone)[27]. In addition to the serum 17OHP concentrations presented in the section hormonal analysis, we also assessed metabolic control by a subjective rating, ~~of metabolic control~~ of the local examining physician at 5 different time points: at diagnosis, at the age of 9 months, at Tanner stage 2, at age 16 years and at study inclusion, using the following scores: "poor", "moderate", "good", "excellent" or "unknown".

Statistical Analysis

SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) was used for all analyses. Descriptive analyses were performed for all variables. Depending on normality, mean and 95% confidence intervals (95%CI) or median and interquartile ranges (IQR) were calculated. We compared patients with values below or above reference range to patients with normal values (within the reference range). Odds ratios (OR) with 95%CI were calculated if at least 3 cases were present in both subgroups. If any cell count in the contingency table was zero, OR and 95%CI were calculated manually by using a continuity correction (+0.5 in each cell).

Missing data were evaluated for each variable and the total number of participants in a particular analysis was reported exactly. Analysis of the variables was ~~only~~ performed only if ~~when~~ the number of participants was ≥ ~~at least~~ 25% of the total cohort of male patients with CAH.

Three patients were excluded from part of the analyses as they received testosterone substitution, which directly affects testosterone and gonadotropin concentrations. Two of these patients had data on TART available; these are described in the results section~~, but were otherwise excluded from further analyses~~. Furthermore, we excluded 22 patients with missing genotype information and 2 patients with 11β-hydroxylase deficiency from all comparative analyses.

Results

General characteristics of the male CAH cohort

A total of 121 male patients were included in the CAH cohort in the dsd-LIFE study. General characteristics are shown in Table 1. The median age of the study population was 28 years (IQR: 18.5-37.5, range 16-68). Mean height was 170.7 (95%CI: 169.3-172.0) cm and median BMI was 25.6 (IQR: 22.0-29.2) kg/m^2 (data available for 119 patients). Nearly ~~Almost~~ all patients had 21OHD (119/121), ~~of which~~ and 97 were confirmed by molecular genetic analysis ~~and 22 were based on phenotype alone~~. The remaining 2 patients had 11β-hydroxylase deficiency. Among the 97 patients with genetically confirmed 21OHD, ~~Genotype groups null, A, B, and C contained 19.8%~~ 24.7% were classified as genotype null, ~~30.6%~~ 38.1% as genotype A, ~~27.3%~~ 34.0% as genotype B, and ~~2.5%~~ 3.1% as genotype C. ~~of the 97 patients with genotyping results. The majority of patients (62.0%) were classified as having the SW form of CAH, 31.4% had the SV form and 4.1% had the NC form.~~ Glucocorticoids were used by 116 (95.9%) patients, most commonly hydrocortisone, followed by prednisone or prednisolone, and dexamethasone. Fludrocortisone was used by 86 patients (71.1%). The patients' education was intermediate or high in 54.5%, and 22.3% ~~of the participants~~, respectively. Furthermore, 54.6% of the patients were in a relationship at the time of study.

We analyzed all variables mentioned in the method section, but we only present in detail the data that differed between the analyzed groups (no overlap in the confidence intervals). In the following sections we will present data regarding hormone concentrations, semen analysis and TART.

Hormone concentrations

Univariate descriptive analyses of hormone concentrations were performed. The proportion of patients with normal, decreased or increased serum testosterone, LH, FSH, inhibin B, AMH, and SHBG concentrations is illustrated in Figure 1A. Hormone concentrations were below the reference range in 19/97 (19.6%: testosterone), 8/43 (18.6%: inhibin B), 12/90 (13.3%: LH), 9/90 (10.0%: FSH), and

1/69 (1.4%: SHBG) of the participants. SHBG concentrations were above the reference range in 14.5% (10/69).

Table 2 ~~shows~~ compares testosterone and gonadotropin concentrations in all patients with data on T, LH, and, FSH available. ~~that in p~~ Seven patients (50%) with decreased testosterone concentrations ~~(19/83), 8 (42.1%)~~ had decreased gonadotropins, while ~~10~~ 6 (~~52.6~~ 42.9%) had normal LH and FSH concentrations, and 1 (~~5.3~~ 7.1%) patient had gonadotropin concentrations above reference range. Normal testosterone concentrations were found in ~~64/83~~ 55/69 (~~77.1~~ 79.7%) patients, ~~50~~ 44 (~~78.1~~ 80.0%) of whom had normal gonadotropin concentrations, whereas ~~9~~ 7 (~~14.1~~ 12.7%) had increased, and ~~5~~ 4 (7.3~~8~~%) had decreased concentrations. Decreased testosterone concentrations were clearly associated with decreased LH and/or decreased FSH concentrations (OR ~~8.0~~ 12.8, 95%CI: 2.9 - ~~2 - 29.6~~ 57.3).

A serum ~~An~~ AD/T ratio was calculated in 49 patients, 22 of whom (44.9%) had an AD/T ratio ≥1. Ten patients (45.5%) with an AD/T ≥1 had decreased gonadotropins, while 11 (50.0%) patients had normal gonadotropins and only 1 (4.5%) patient had increased gonadotropins. Normal AD/T ratios were found in 27/49 (55.1%) patients, 21 of whom had normal gonadotropin concentrations (77.8%), 5 had increased concentrations, but none had decreased gonadotropin concentrations. ~~was found in 7/8 patients (87.5%) with decreased testosterone and gonadotropins, while 4/5 patients (80.0%) with normal testosterone and decreased gonadotropins had an AD/T ratio ≥1. Moreover, 5/10 patients (50.0%) with decreased testosterone and normal gonadotropins had an AD/T ratio ≥1, whereas this was seen in only 11/50 patients (22.0%) with normal testosterone and gonadotropins.~~ An AD/T ratio ≥1 was strongly associated with decreased LH and/or decreased FSH concentrations (OR ~~16.8~~ 39.3, 95%CI: 2.1~~0~~ - ~~142.5~~ 732.4).

Semen analysis

Semen analysis was performed in approximately one third of the patients (Figure 1B). Normal values for all known (at least 3 out of 5) semen parameters (normozoospermia) were seen in 11/39 patients

in which semen analysis was performed. Sperm concentration, motility, and volume were below the normal ranges in 38.5% (15/39), 35.1% (13/37), and 25.6% (10/39) of the patients, respectively, while morphology and vitality were both impaired in 14.3% (4/28 and 2/14) of the patients. Five of 8 patients (62.5%) with decreased testosterone and gonadotropin concentrations underwent semen analysis, with 4 (80.0%) of them showing abnormal semen parameters (Table 3). In only 2/10 patients with decreased testosterone, but normal gonadotropin concentrations, semen analysis was performed and both had decreased sperm concentrations (7.0 and 10.0 x10^6/mL). No statistically significant associations were found ~~(data not shown)~~.

Testicular adrenal rest tumors

TARTs were visualized by ultrasound or MRI at cross-sectional investigation in 28/68 patients. For 1 patient, the diagnosis was based on retrospective histology data. Furthermore, retrospective imaging data were available for 11 men: TARTs were present in 10 of these individuals. ~~So, in~~ In the total population screened, TARTs were present in 39/80 patients (48.8%) of which 34 were bilateral TARTs (87.2%). Documented retrospective TARTs at age 16 years were reported in 16/30 patients (53.3%), all of which were bilateral. In only 2/16 patients (12.5%) with TART reported to be present at age 16, TART was no longer observed during the cross-sectional investigation: one patient was misdiagnosed with TART as it appeared to be a varicocele, and in the other patient TART (size 2 mm) disappeared after treatment with prednisone. This patient was still considered as a ~~TART~~ patient with TART in all analyses.

Comparison of patients with and without TART

Table 4 shows associations of TART with various variables in the ~~78~~ 68 patients with gonadal imaging data (12 patients were excluded due to testosterone substitution, 11β-hydroxylase deficiency or unconfirmed 21-hydroxylase deficiency), comprising ~~37~~ 33 patients with and ~~41~~ 35 without TARTs. Genotype was ~~clearly~~ associated with the presence of TART: The null genotype group had the highest prevalence of TART (14/18: 77.8%), while the prevalence was 10/27 (37.0%) for genotype group A,

and 7/21 (33.3%) for genotype group B. The odds of having TART in the null genotype group was 6.0 [1.5-23.1] and 7.0 [1.7-29.4] times higher compared to the genotype groups A and B, respectively. TARTs were also present in both men in the genotype C group, and also in 1 CYP11B1-deficient patient (the other CYP11B1 patient did not undergo assessment for TART). The OR of having TART when having ~~an~~ a serum androstenedione ~~level~~ concentration above the upper limit of normal at the time of the cross-sectional investigation was 3.6 ~~3~~ [1.0 - ~~11.2~~ 12.7]. Similar associations were found for serum 17OHP at the cross-sectional investigation, with an OR of ~~6.4~~ 28.0 [~~1.7~~ 3.1 - ~~24.7~~ 252.5] for having TART when 17OHP concentrations were more than twice the upper level of the reference range, and an OR of ~~4~~ 18.7 [~~1.3~~ 2.2 - 158.1 ~~16.5~~] when these concentrations were above the reference range compared to concentrations within the reference range.

Paternity

Data on paternity were available for 113 of the 121 patients, 26 (23.0%) of whom (age range 26-68 years) had fathered a total of 43 children. Three couples had used assisted reproductive techniques (ART) resulting in 4/43 children. One of the men who had used ART had decreased testosterone concentrations, while another had increased FSH, decreased sperm concentration, and TART. No information was available about the third patient who had used ART. ~~One man with impaired semen motility, increased FSH concentrations, and TART had adopted a child.~~

Discussion

This unique and relatively large European multicenter study shows that gonadal dysfunction is a common complication in male patients with CAH. Approximately half of the patients were affected by endocrine disturbances of the HPG axis at an adult age and TARTs were present in approximately half of the patients as well.

The difficulty in diagnosing hypogonadism in men with CAH is related to the fact that testosterone measured in serum is a mixture of testosterone of gonadal and adrenal origin [25, 28]. Circulating

testosterone in male patients with well-controlled CAH is predominantly derived from testicular production, but when there is poor hormonal control, a relevant contribution arises from adrenal steroidogenesis. Until now, no method is able to discriminate between testosterone derived from the testes or the adrenal gland. Therefore, it has been suggested to use the serum AD/T ratio in male patients with CAH, as this precursor steroid is elevated in serum when serum androgens are predominantly of adrenal origin [25]. Our data point toward an association ~~confirmed a stronger association~~ between an AD/T ratio ≥1 (testosterone mainly of adrenal origin) and decreased LH and/or decreased FSH concentrations ~~compared to testosterone concentrations alone~~, suggesting that adrenal androgens in men with CAH contribute to the suppression of gonadotropins. In approximately half of the patients, either aberrant testosterone or AD/T ratios, or aberrant gonadotropin concentrations, or a combination of both were found. In previous studies, the reported prevalence of endocrine HPG axis disturbances ranged from 20% to 52% [5-7, 9, 10]. However, only 1 other ~~report~~ study provided ~~had~~ information on testosterone and gonadotropin concentrations in each patient, and also indicated ~~endocrine disturbances~~ hypogonadism in approximately half of the patients [6]. We recommend to ~~including~~ include the evaluation of the AD/T ratio in the regular follow-up ~~androstenedione measurements in the gonadal evaluation~~ of male patients with CAH ~~to calculate the AD/T ratio,~~ and interpret this ratio in combination with gonadotropin concentrations in order to detect a disturbance of the HPG axis. Our study ~~did~~ does not include data ~~information~~ on 11-oxygenated androgens, that are generated through conversion of androstenedione, and are reported to be elevated ~~concentrations are found~~ in patients with CAH [29, 30]. Recent studies indicate that 11-oxygenated androgens are almost entirely derived from the 11beta-hydroxylation of androstenedione in the adrenal, and as they are potent androgens they can contribute to suppression of the HPG axis[31]. However, their exact role in the evaluation of ~~However, their associations with~~ hormonal control and gonadal function in men with CAH has to be established in further studies. Serum AMH and inhibin B are also used as markers for male fertility [32]. However, ~~literature already showed~~ it has been demonstrated that serum AMH concentrations do not

correlate with sperm concentration and other male fertility parameters [33]. Serum inhibin B, a marker of Sertoli cell function, is known to correlate with spermatogenesis in healthy men [34] and was decreased in ~~18.3%~~ 18.6% of our cohort. Semen quality, assessed in one third of the study cohort, was reduced in 40% of the men. Except for the study of Urban et al. [3], all other studies on fertility in male patients with CAH showed decreased sperm concentrations ranging from 47.8% to 66% [4-7, 9, 10]. More strikingly, in all studies only half of the participants participated in semen analysis. Taken together, these data indicate the need for ~~Therefore,~~ increased awareness on fertility status in patients with CAH, and to start ~~is needed. We recommend~~ performing semen analysis and gonadal function biomarkers assessment ~~as early as possible in~~ from adolescence on, in order to detect disturbances early and allow semen preservation ~~to be able to preserve semen~~ for later fertility purposes.

Data from our cohort indicate, in agreement with previous studies ~~The prevalence of TART in the present patient cohort was 48.8%, confirming previous reports that TART is a common complication in male patients with CAH~~ [4-10, 12-22], that TART is a common complication in males with CAH (with a prevalence of 48.8%) and can have onset as early as in adolescence. In fact, ~~Strikingly,~~ 14 patients with TART at the time of the dsd-LIFE study already had TART at the age of 16 years. TARTs disappeared on ~~at 16 years were no longer detectable following~~ treatment with prednisone in only 1 patient, thus indicating~~. This could indicate~~ that complete regression of TART might only be achieved in a small proportion of the patients. Hence, prevention of the development of TART should be pursued, by optimizing treatment strategies already in childhood. Current standard of care does not include imaging of testes, however we recommend incorporating testicular ultrasound in routine clinical practice.

In contrast to previous studies~~, several studies did not find an association between CAH severity and TART~~ [4, 9, 10], we observed an association between the *CYP21A2* genotype and the presence of TARTs, with the prevalence of this complication being ~~was~~ highest in men with the null *CYP21A2* genotype.

This ~~likely confirms~~ supports the current perception that TARTs are more frequently observed in patients with a more severe form of CAH, as these patients are exposed to higher concentrations of ACTH, already *in utero*, which is thought to be a possible causative factor for TART development [6, 7, 15, 22]. However, a clinically relevant finding in this study is that TARTs occurs even in less severe forms of 21OHD. In fact, in ~~. In~~ our study, 2 patients in genotype group C ~~with NC CAH~~ (both compound heterozygous for deletion and P30L mutation) had TARTs. In our current dataset, we could not find an association between genotype and semen quality or genotype and hypogonadism. ~~Both patients were compound heterozygous (deletion + P30L mutation). Only 1 patient in our cohort had the typical NC mutation, i.e. the V281 mutation (V281/I2Splice). No TARTs were detected in this patient.~~

~~In our study, we~~ We found an association between increased 17OHP concentrations at cross-sectional data assessment and the presence of TART. Although a single 17OHP measurement may not be representative of overall metabolic control, these results could be interpreted as a possible indicator of the patient´s metabolic control in the recent past. Therefore, our results seem to be in accordance with literature reporting higher TART prevalence in patients with poor hormonal control compared to patients with adequate hormonal control [5, 7, 13, 35-38]. The association between increased androstenedione concentrations at cross-sectional data assessment and the presence of TARTs adds evidence to this pathophysiologic concept, even if the AD/T ratios were not clearly associated with TART within this subgroup of patients. Primary gonadal dysfunction may be suggested by raised FSH concentrations. In our dataset, 10 patients (11.1%) had elevated FSH concentrations. Seven of these patients had data on the presence of TART, and 4 had evidence of TART. King et al. found that testicular failure was a consequence of TART in the majority of cases [10]. However, our data are limited and do not allow firm conclusions concerning this issue. ~~We cannot confirm the findings of King as we have only very limited data available~~.

Despite this being the first international multicenter study describing gonadal function in male patients with CAH, the study also has some limitations. All centers included in this consortium are

tertiary care centers, therefore it is possible that the patient groups were selected and that the patients included were more severely affected. Furthermore, serum hormone concentrations were not measured centrally, but in various centers with a range of different assays. Accounting for this fact, only range variables were used in the data analyses. The median BMI in our patient cohort was 25.6 kg/m^2 (range 22.0-29.2), which is slightly overweight. It has been demonstrated that excess of total and abdominal body fat could represent one cause of fertility impairment in men with CAH [25]. Serum total testosterone can be decreased in patients with obesity, as a result of the decreased serum concentration of SHBG. In case of increased serum SHBG (induced by hepatitis, hyperthyroidism, or a genetic variant), total testosterone may be increased. Ideally, free testosterone should be measured in these cases, but this requires complex equilibrium dialysis [39]. Free testosterone can also be calculated from ~~the level of~~ total testosterone, SHBG, and albumin concentrations, but it is crucial that the results of such calculations are compared with the normal range of each separate laboratory. Such data were not available. We are aware that assessment of fertility by paternity numbers in our study was ~~is~~ incomplete, as many other factors, ~~of which~~ including female fertility, ~~are important as well. However, these data~~ were not available. Furthermore, participation in the medical examination was not ~~obligatory~~ compulsory for study inclusion. This may have led to even more selection, especially concerning the ultrasound examination and semen analysis. It is likely that only the very motivated patients and the more severely affected patients consented to these additional examinations. Due to the resulting low numbers of available data, multivariable logistic regression analyses were not possible.

In summary, impaired gonadal function is common in adult men with CAH. This is indicated by the presence of TART and/or hypogonadotropic or hypergonadotropic hypogonadism. The risk of TART is highest in men with the most severe ~~forms of~~ enzyme deficiencies underlying CAH. Our data suggest that an ~~An~~ association with poor previous hormonal control is likely but ~~has to be confirmed~~ requires confirmation by ~~further~~ prospective studies. Determination of the serum AD/T ratio, in addition to serum concentrations of testosterone, androstenedione, LH, and FSH may help to differentiate

between testicular and adrenal androgens in male patients with CAH and to ~~estimate the degree~~ diagnose ~~of~~ gonadal dysfunction. Routinely performed semen analysis, measurement of serum inhibin B, and testicular ultrasound investigation already in adolescence are recommended to detect upcoming reproductive problems and to allow for fertility preserving measures, such as sperm banking.

Disclosure

Funding

dsd-LIFE - The work leading to these results received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 305373. The following author received additional funding: NR: Else Kröner-Fresenius Stiftung (Grant 2011-EKMS.21), and the European Community (Marie Curie European Reintegration Grant PERG-GA-2010-268270).

~~Declaration~~ Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Acknowledgements

We are grateful to the participants of dsd-LIFE and to all of the study centers for their enthusiasm and dedication in contacting potential participants and collecting high-quality data. We especially thank the support groups in the different countries for their help. For an overview of all contributors we refer to our study protocol [23].

References

1. El-Maouche D, Arlt W & Merke DP. Congenital adrenal hyperplasia. *Lancet* 2017.
2. Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, Meyer-Bahlburg HF, Miller WL, Montori VM, Oberfield SE, Ritzen M, White PC & Endocrine S. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010 **95** 4133-4160.
3. Urban MD, Lee PA & Migeon CJ. Adult height and fertility in men with congenital virilizing adrenal hyperplasia. *N Engl J Med* 1978 **299** 1392-1396.

4. Falhammar H, Nystrom HF, Ekstrom U, Granberg S, Wedell A & Thoren M. Fertility, sexuality and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia. *Eur J Endocrinol* 2012 **166** 441-449.

5. Cabrera MS, Vogiatzi MG & New MI. Long term outcome in adult males with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2001 **86** 3070-3078.

6. Stikkelbroeck NM, Otten BJ, Pasic A, Jager GJ, Sweep CG, Noordam K & Hermus AR. High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2001 **86** 5721-5728.

7. Reisch N, Flade L, Scherr M, Rottenkolber M, Pedrosa Gil F, Bidlingmaier M, Wolff H, Schwarz HP, Quinkler M, Beuschlein F & Reincke M. High prevalence of reduced fecundity in men with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2009 **94** 1665-1670.

8. Arlt W, Willis DS, Wild SH, Krone N, Doherty EJ, Hahner S, Han TS, Carroll PV, Conway GS, Rees DA, Stimson RH, Walker BR, Connell JM, Ross RJ & United Kingdom Congenital Adrenal Hyperplasia Adult Study E. Health status of adults with congenital adrenal hyperplasia: a cohort study of 203 patients. *J Clin Endocrinol Metab* 2010 **95** 5110-5121.

9. Bouvattier C, Esterle L, Renoult-Pierre P, de la Perriere AB, Illouz F, Kerlan V, Pascal-Vigneron V, Drui D, Christin-Maitre S, Galland F, Brue T, Reznik Y, Schillo F, Pinsard D, Piguel X, Chabrier G, Decoudier B, Emy P, Tauveron I, Raffin-Sanson ML, Bertherat J, Kuhn JM, Caron P, Cartigny M, Chabre O, Dewailly D, Morel Y, Touraine P, Tardy-Guidollet V & Young J. Clinical Outcome, Hormonal Status, Gonadotrope Axis, and Testicular Function in 219 Adult Men Born With Classic 21-Hydroxylase Deficiency. A French National Survey. *J Clin Endocrinol Metab* 2015 **100** 2303-2313.

10. King TF, Lee MC, Williamson EE & Conway GS. Experience in optimizing fertility outcomes in men with congenital adrenal hyperplasia due to 21 hydroxylase deficiency. *Clin Endocrinol (Oxf)* 2016 **84** 830-836.

11. Falhammar H, Frisen L, Norrby C, Almqvist C, Hirschberg AL, Nordenskjold A & Nordenstrom A. Reduced Frequency of Biological and Increased Frequency of Adopted Children in Males With 21-Hydroxylase Deficiency: A Swedish Population-Based National Cohort Study. *J Clin Endocrinol Metab* 2017 **102** 4191-4199.

12. Jaaskelainen & Voutilainen R. Long-term outcome of classical 21-hydroxylase deficiency: diagnosis, complications and quality of life. *Acta Paediatr* 2000 **89** 183-187.

13. Kamoun M, Mnif MF, Charfi N, Ben Naceur B, Mnif F, Rekik N, Mnif Z, Sfar MH, Sfar MT, Hachicha M, Ben Salem A, Keskes LA & Abid M. Fertility outcome in male and female patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Middle East Fertility Society Journal* 2014 **19** 89-95.

14. Mouritsen A, Jorgensen N, Main KM, Schwartz M & Juul A. Testicular adrenal rest tumours in boys, adolescents and adult men with congenital adrenal hyperplasia may be associated with the CYP21A2 mutation. *Int J Androl* 2010 **33** 521-527.

15. Nermoen I, Rorvik J, Holmedal SH, Hykkerud DL, Fougner KJ, Svartberg J, Husebye ES & Lovas K. High frequency of adrenal myelolipomas and testicular adrenal rest tumours in adult Norwegian patients with classical congenital adrenal hyperplasia because of 21-hydroxylase deficiency. *Clin Endocrinol (Oxf)* 2011 **75** 753-759.

16. Delfino M, Elia J, Imbrogno N, Argese N, Mazzilli R, Toscano V & Mazzilli F. Testicular adrenal rest tumors in patients with congenital adrenal hyperplasia: prevalence and sonographic, hormonal, and seminal characteristics. *J Ultrasound Med* 2012 **31** 383-388.

17. Finkielstain GP, Kim MS, Sinaii N, Nishitani M, Van Ryzin C, Hill SC, Reynolds JC, Hanna RM & Merke DP. Clinical characteristics of a cohort of 244 patients with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2012 **97** 4429-4438.

18. Pierre P, Despert F, Tranquart F, Coutant R, Tardy V, Kerlan V, Sonnet E, Baron S, Lorcy Y, Emy P, Delavierre D, Monceaux F, Morel Y & Lecomte P. Adrenal rest tissue in gonads of patients

with classical congenital adrenal hyperplasia: multicenter study of 45 French male patients. *Ann Endocrinol (Paris)* 2012 **73** 515-522.

19. Reisch N, Rottenkolber M, Greifenstein A, Krone N, Schmidt H, Reincke M, Schwarz HP & Beuschlein F. Testicular adrenal rest tumors develop independently of long-term disease control: a longitudinal analysis of 50 adult men with congenital adrenal hyperplasia due to classic 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 2013 **98** E1820-1826.

20. Dudzinska B, Leubner J, Ventz M & Quinkler M. Sexual well-being in adult male patients with congenital adrenal hyperplasia. *Int J Endocrinol* 2014 **2014** 469289.

21. Bachelot A, Golmard JL, Dulon J, Dahmoune N, Leban M, Bouvattier C, Cabrol S, Leger J, Polak M & Touraine P. Determining clinical and biological indicators for health outcomes in adult patients with childhood onset of congenital adrenal hyperplasia. *Eur J Endocrinol* 2015 **173** 175-184.

22. Claahsen-van der Grinten HL, Sweep FC, Blickman JG, Hermus AR & Otten BJ. Prevalence of testicular adrenal rest tumours in male children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Eur J Endocrinol* 2007 **157** 339-344.

23. Rohle R, Gehrmann K, Szarras-Czapnik M, Claahsen-van der Grinten H, Pienkowski C, Bouvattier C, Cohen-Kettenis P, Nordenstrom A, Thyen U, Kohler B & dsd Lg. Participation of adults with disorders/differences of sex development (DSD) in the clinical study dsd-LIFE: design, methodology, recruitment, data quality and study population. *BMC Endocr Disord* 2017 **17** 52.

24. Krone N & Arlt W. Genetics of congenital adrenal hyperplasia. *Best Pract Res Clin Endocrinol Metab* 2009 **23** 181-192.

25. Auchus RJ. Management considerations for the adult with congenital adrenal hyperplasia. *Mol Cell Endocrinol* 2015 **408** 190-197.

26. Cooper TG, Noonan E, von Eckardstein S, Auger J, Baker HW, Behre HM, Haugen TB, Kruger T, Wang C, Mbizvo MT & Vogelsong KM. World Health Organization reference values for human semen characteristics. *Hum Reprod Update* 2010 **16** 231-245.

27. Claahsen-van der Grinten HL, Stikkelbroeck NM, Otten BJ & Hermus AR. Congenital adrenal hyperplasia--pharmacologic interventions from the prenatal phase to adulthood. *Pharmacol Ther* 2011 **132** 1-14.

28. Rohayem J, Tuttelmann F, Mallidis C, Nieschlag E, Kliesch S & Zitzmann M. Restoration of fertility by gonadotropin replacement in a man with hypogonadotropic azoospermia and testicular adrenal rest tumors due to untreated simple virilizing congenital adrenal hyperplasia. *Eur J Endocrinol* 2014 **170** K11-17.

29. Jones CM, Mallappa A, Reisch N, Nikolaou N, Krone N, Hughes BA, O'Neil DM, Whitaker MJ, Tomlinson JW, Storbeck KH, Merke DP, Ross RJ & Arlt W. Modified-Release and Conventional Glucocorticoids and Diurnal Androgen Excretion in Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab* 2017 **102** 1797-1806.

30. Turcu AF, Mallappa A, Elman MS, Avila NA, Marko J, Rao H, Tsodikov A, Auchus RJ & Merke DP. 11-Oxygenated Androgens Are Biomarkers of Adrenal Volume and Testicular Adrenal Rest Tumors in 21-Hydroxylase Deficiency. *J Clin Endocrinol Metab* 2017 **102** 2701-2710.

31. Pretorius E, Arlt W & Storbeck KH. A new dawn for androgens: Novel lessons from 11-oxygenated C19 steroids. *Mol Cell Endocrinol* 2017 **441** 76-85.

32. Lahlou N, Bouvattier C, Linglart A, Rodrigue D & Teinturier C. [The role of gonadal peptides in clinical investigation]. *Ann Biol Clin (Paris)* 2009 **67** 283-292.

33. Kucera R, Ulcova-Gallova Z, Windrichova J, Losan P & Topolcan O. Anti-Mullerian hormone in serum and seminal plasma in comparison with other male fertility parameters. *Syst Biol Reprod Med* 2016 **62** 223-226.

34. Andersson AM, Petersen JH, Jorgensen N, Jensen TK & Skakkebaek NE. Serum inhibin B and follicle-stimulating hormone levels as tools in the evaluation of infertile men: significance of

adequate reference values from proven fertile men. *J Clin Endocrinol Metab* 2004 **89** 2873-2879.

35. Claahsen-van der Grinten HL, Dehzad F, Kamphuis-van Ulzen K & de Korte CL. Increased prevalence of testicular adrenal rest tumours during adolescence in congenital adrenal hyperplasia. *Horm Res Paediatr* 2014 **82** 238-244.

36. Aycan Z, Bas VN, Cetinkaya S, Yilmaz Agladioglu S & Tiryaki T. Prevalence and long-term follow-up outcomes of testicular adrenal rest tumours in children and adolescent males with congenital adrenal hyperplasia. *Clin Endocrinol (Oxf)* 2013 **78** 667-672.

37. Bachelot A, Plu-Bureau G, Thibaud E, Laborde K, Pinto G, Samara D, Nihoul-Fekete C, Kuttenn F, Polak M & Touraine P. Long-term outcome of patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Horm Res* 2007 **67** 268-276.

38. Poyrazoglu S, Saka N, Agayev A & Yekeler E. Prevalence of testicular microlithiasis in males with congenital adrenal hyperplasia and its association with testicular adrenal rest tumors. *Horm Res Paediatr* 2010 **73** 443-448.

39. Bhasin S, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM & Task Force ES. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010 **95** 2536-2559.

Figure legends

Figure 1: Hormone concentrations (A) and semen quality (B) in male patients with congenital adrenal hyperplasia ~~to assess gonadal function~~. Stacked bars represent percentage of patients within a category. Numbers in the bars represent the specific number of patients within a category, while the total number of patients included in this analysis is stated underneath the x-axis. **A)** Hormone concentrations of each patient were measured in the local hospital and compared to the hospitals standard reference ranges. **B)** Semen analysis was performed and scored according to World Health Organization 2010 criteria [26]: sperm concentration, motility, morphology, and vitality, and semen volume were assessed. Abbreviations: AMH, anti-Müllerian hormone; INHB, inhibin B; N, number of patients; T, testosterone.